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Pharmacodynamics and dermatopharmacokinetics of betamethasone 17-valerate: Assessment of topical bioavailability

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Short title: Topical drug bioavailability

Abbreviations:

AARC Area above the response curve

AUC Area under the curve

BMV Betamethasone 17-valerate

D/L² Diffusivity parameter

DPK Dermatopharmacokinetic

K Stratum corneum-vehicle partitioning coefficient

LMO Light mineral oil

MCT Medium chain triglycerides

ME Microemulsion

SC Stratum corneum

TCL Transcutol[®]

TG Topical glucocorticoids

Summary

Background: The bioavailability of most topically-delivered drugs is difficult to quantify, but is generally believed to be very low. With the exception of the vasoconstrictor assay for corticosteroids, no methodology to quantify the rate and extent of drug delivery to the skin has been validated. Recent research has examined the dermatopharmacokinetic (DPK) technique, which is based on stratum corneum (SC) tape-stripping.

Objective: To compare the *in vivo* bioavailability of different topical formulations of betamethasone 17-valerate (BMV) using the vasoconstrictor assay and the DPK method.

Methods: BMV was formulated in different vehicles and the drug concentration was adjusted to either (i) equal thermodynamic activity, or (ii) a range of values up to that corresponding to 80% of maximum thermodynamic activity. Vasoconstriction, an accepted and widely-used method to determine bioavailability and bioequivalence of topical steroids, was quantified with a chromameter over 24 hours post-removal of the formulation. Drug uptake into the SC was assessed by tape-stripping.

Results: BMV at the same thermodynamic activity in different vehicles provoked similar skin blanching responses, while DPK profiles distinguished between the formulations. Further, skin blanching responses and drug uptake into the SC clearly depended upon the absolute BMV concentration applied. However, while the saturable nature of the pharmacodynamic response was clear, the tape-stripping method showed distinguished unequivocally between different formulations and different concentrations.

Conclusions: The DPK approach offers a reliable metric with which to quantify transfer of drug from the vehicle to the SC, and may be useful for topical bioavailability and bioequivalence determinations.

Key words: corticosteroids, dermatopharmacokinetics, topical bioavailability, topical bioequivalence, vasoconstrictor assay

The efficiency of topical delivery is notoriously inefficient, with typical bioavailabilities of only a few percent of the applied dose. Rational development of better formulations requires better creativity and better methods with which to quantify bioavailability (BA) and bioequivalence (BE) of drug delivery to a target in the skin. Topical BA/BE evaluation usually requires clinical trials, which are invasive, relatively insensitive, time-consuming and costly. In the case of topical glucocorticoids (TG), however, BA/BE can be assessed using the vasoconstrictor assay. In 1995, the U.S. Food and Drug Administration (FDA) published a Guidance document for testing BE of different TG formulations¹. This Guidance consists of a pilot study to explore the dose duration-response relationship of a reference listed drug (RLD) and to determine the appropriate dose duration for use in a subsequent pivotal test, which then compares the *in vivo* response of the test product with that of the RLD to document whether BE has, or has not, been achieved.

Apart from TG, for all other topical drugs, however, the vasoconstrictor assay is not useful to document BA/BE and, at the moment, clinical trials are the only option. However, in 1998, evolving from the first quantitative stratum corneum (SC) tape-stripping experiments²⁻⁵, the FDA proposed an alternative, potentially more generally applicable, technique instead: the dermatopharmacokinetic approach (DPK), analogous to the pharmacokinetic method of oral drug BA/BE assessment^{6,7}. The DPK approach evaluates topically applied drug levels in the SC, the outermost layer of the skin, as a function of time post-application and post-removal of the formulation. The Draft Guidance⁶ allows the assessment of both drug uptake into and drug elimination from the SC. At specific times, layers of the SC are sequentially removed at the treated site with adhesive tapes and the total amount of drug is subsequently analyzed therein. From the DPK profile of drug mass in the SC as a function of time, pharmacokinetic parameters such as the area under the curve (AUC), the maximum amount drug in the tape-strips

(A_{\max}) and the time (T_{\max}) at which A_{\max} is attained are deduced and used to characterize the local BA.

The DPK method assumes that SC drug levels are directly related to those in the viable epidermis and/or dermis, as the SC is typically the rate-determining barrier to percutaneous absorption. In other words, it is hypothesized that the rate and extent of drug disposition in the SC will reflect that achieved at target sites which are further into the skin. Indeed, there have been experiments using commercially available TG products in which the drug uptake phase into the SC using tape-stripping have been favorably compared with the pharmacodynamic response⁸⁻¹¹. However, this Draft Guidance was withdrawn in 2002, mainly because of doubts regarding reproducibility, flaws resulting from the similar design of the approach to oral BE assessment, and criticism that quantification of the amount of SC removed should be better controlled¹².

As a consequence, a critical re-evaluation of the DPK method is in progress, with a clear objective being to validate a refined approach. Important progress has been made with respect to quantification and standardization of the amount of SC removed during tape-stripping^{13,14} such that drug concentration profiles across the membrane can now be expressed on the same scale: that is, as a function of the relative position within the SC¹⁵. Equally, DPK parameters, characterizing drug partitioning and diffusivity into and through the SC, can be deduced and used to quantify, respectively, the extent and rate of drug delivery. These advances have been illustrated for terbinafine¹⁶⁻¹⁸ and for ibuprofen¹⁹⁻²² delivered from different vehicles.

The goal of this study was to explore the challenge of validating the DPK methodology by comparing the assessment of the topical BA of betamethasone 17-valerate (BMV) using the vasoconstrictor assay and the tape-stripping approach. With respect to the latter method, the protocol specified in the Draft Guidance⁶ was compared to alternative procedures introduced more recently¹⁸⁻²². The sensitivity of the

techniques, and their ability to discriminate between different formulations, was examined as a function of the applied drug concentration and thermodynamic activity (a measure of the drug's so-called 'leaving tendency' from the vehicle and is related to its solubility).

Materials & Methods

Formulations

Betamethasone 17-valerate (BMV) (Crystal Pharma, Boecillo, Spain) was dissolved in (i) the reference vehicle, medium chain triglycerides (MCT) (Mygliol 812N, Synopharm, Barsbüttel, Germany), and in (ii) light mineral oil (LMO) (Synopharm), (iii) the microemulsion Mikro 100[®] (ME) (Sebapharma, Boppard, Germany), and (iv) Transcutol[®] P (TCL) (Gattefossé, Saint Priest, France), as test vehicles. The components of the ME were aqua, polysorbate 20, polyglyceryl-6 dioleate, ethylhexyl cocoate, PEG-8 caprylic/capric glycerides, denatured alcohol, tocopheryl acetate, ectoin, panthenol, centella asiatica, ethoxydiglycol oleate, sodium lactate, parfum and phenoxyethanol. To avoid spreading of the formulations on the skin, either 15% (w/w) polypropylene (Sigma-Aldrich, Steinheim, Germany) or 10% (w/w) Aerosil[®] 200 (Sigma-Aldrich) was used to thicken the vehicles into semi-solid gels. The formulations studied, although not as complex as many commercialized products, provided reasonable models for those in clinical use and were able to solubilize BMV over a wide range of concentrations.

The saturation level ($C_{s,v}$) of BMV in each vehicle was determined by stirring a suspension of the drug in the liquid vehicles at 32°C until equilibrium was attained (about 72 hours). The samples were centrifuged, diluted either with acetonitrile alone or with acetonitrile/water 60:40 (v/v) and analyzed by liquid chromatography (see method below). Because of the incompatibility of LMO with acetonitrile, the saturation level of BMV in this excipient was determined using the iterative method of visual clouding. The experiments were performed in triplicate.

In the first part of the study, the BMV concentration was adjusted to 80% of saturation in each vehicle to provide the drug at equivalent thermodynamic activity (Table 1). This means that the “leaving tendency” of the drug from these formulations

was 80% of that from a vehicle in which BMV is present as a suspension, i.e., a saturated solution (the case for many of the ointment and cream products in clinical use).

Table 1: Saturation level ($C_{s,v}$) of BMV in different vehicles at 32°C (mean \pm SD, $n = 3$).

Vehicle	$C_{s,v}$ [mg mL ⁻¹]
LMO	0.0021 \pm 0.0003
MCT	2.1 \pm 0.2
ME	11.7 \pm 0.4
TCL	126 \pm 1.1

In the second component of the investigation, only the MCT and ME vehicles were evaluated and the BMV concentration was adjusted to different degrees of its saturation level (Table 2).

Table 2: Thermodynamic activities of BMV, expressed as degree of saturation ($C_{s,v}$), and concentrations (C_v) studied in the second set of experiments using ME and MCT formulations only.

Degree of $C_{s,v}$	0.80	0.10	0.05	0.026	0.013	0.0064	0.0032
C_v ME [mg mL ⁻¹]	9.3	1.2	0.6	0.3	0.15	0.07	0.04
C_v MCT [mg mL ⁻¹]	1.7	0.2	-	-	0.027	0.013	-

MCT was chosen as the reference vehicle as it was not expected to have any effect on skin barrier function *per se*²³. BMV was selected as a typical, and frequently

used, class 2 TG²⁴. BMV has a relatively high molecular weight (476.6 Da) and is quite lipophilic ($\log(\text{octanol/water partition coefficient}) = 3.78$).

Vasoconstrictor assay

Twelve healthy Caucasian volunteers (8 female, 4 male), aged 21-55 years, from whom informed consent was obtained, participated in the two studies, which were approved by the Ethics Committee of the University of Leipzig. The study followed the "staggered application with synchronized removal" method of the FDA Guidance "Topical Dermatologic Glucocorticosteroids – *In vivo* Bioequivalence"¹. 250 µl of each formulation were applied in a 1.2 cm diameter Hill Top Chamber[®] (Hill Top Research, Cincinnati, OH, USA) which was affixed with adhesive tape. Although the amount of formulation applied was greater than that which would be used clinically, this infinite 'dose' ensured that drug depletion from the vehicle (either via absorption into the skin or adsorption onto the Hill Top chamber[®]) could be ignored when the data were subsequently interpreted. The drug application sites were on the volar forearm, at least 4 cm from the wrist and 4 cm from the antecubital fossa. Because of the known circadian activity of TG, all formulations were applied only at 0800 hours.

In the first part of the study, LMO, ME and TCL were compared with the reference vehicle, MCT. The formulations, as well the drug-free vehicles as control, were applied to the skin for dose durations of 2, 3, 4, 5 and 6 hours. The chambers were then removed and excess formulation was cleaned off with a dry paper towel. The skin blanching response was assessed with a chromameter (CR-300, Minolta, Ahrensburg, Germany) using the a-scale values at various times up to 24 hours following formulation removal (Figure 1). Baseline readings were taken at all sites prior to the application of the formulations. The a-scale readings for each drug application site were adjusted for the baseline value and the control and expressed as the change in this parameter (Δa). As

the a-scale values decrease with increasing skin blanching, Δa is negative. The degree of response was therefore expressed as the (positive) area above the response curve (AARC) using the trapezoidal rule.

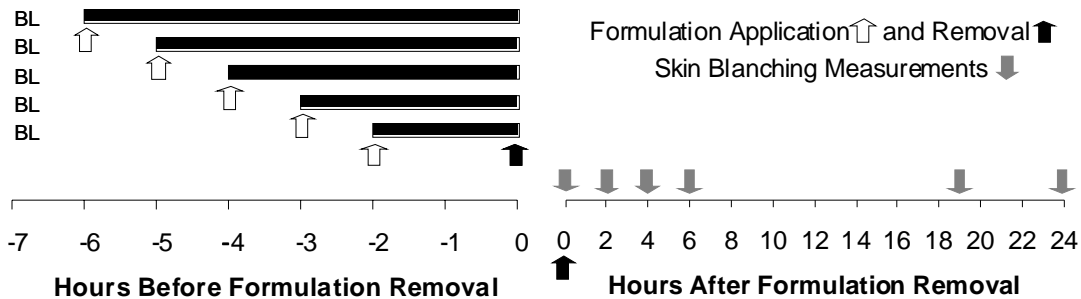


Figure 1: Vasoconstriction experimental protocol. Staggered application with synchronized removal: schematic representation of the pilot study protocol, BL = Baseline¹.

In the second series of experiments, BMV, at different degrees of saturation in MCT and ME (Table 2), were applied to the forearm for 4 hours. This dose duration was chosen to be sure that the blanching response would be quantifiable for all concentrations applied. After removal of the formulations, the skin blanching response was assessed as before over the 24 hour period after formulation removal.

Tape-stripping experiments

Six healthy Caucasian volunteers (4 female, 2 male, 23-41 years) with no history of dermatological disease participated in these measurements, which were approved by the Salisbury and South Wiltshire Research Ethics Committee. Written consent was obtained from all subjects.

A single (and again) infinite dose volume of 600 μL of each BMV formulation was first applied using a 1.8 cm diameter Hill Top Chamber[®] on the volar aspect of the forearm. After a 2-hour application (dose duration), the chambers were removed and excess formulation was cleaned off with a dry paper towel. The application time was selected based on earlier work^{20,21}. Immediately after cleaning, the SC at the treated site was progressively removed by repeated adhesive tape-stripping (Scotch Book Tape, 3M, St. Paul, MN, USA). A piece of polypropylene foil with a predefined hole was placed onto the cleaned, treated skin site and affixed with self-adhesive tape. This template ensured that all tape-stripping procedures took place at the same site (and eliminated any potential problems created by the formulation spreading over the skin). The tape (2.5 x 2.5 cm) was applied over this template, using a constant pressure (140 g cm⁻²) via a weighted roller and then removed. Up to 20 strips were taken from each site, but the SC was never completely removed. To ascertain the remaining skin barrier function, transepidermal water loss (TEWL) measurements were performed (AquaFlux V4.7, Biox Systems Ltd., London, UK) during the stripping procedure, which was stopped when TEWL reached ~ 4 times its initial value or 60 g m⁻² h⁻¹. Each tape was carefully weighed before and after stripping on a 10- μg precision balance (Mettler AT 261, Greifensee, Switzerland) to determine the mass of SC removed. From this mass, the known stripped area, and given that the density of the SC is $\sim 1 \text{ g mL}^{-1}$ ²⁵, it was possible to calculate the SC thickness removed on each tape-strip, and hence the corresponding position within the barrier²⁶. BMV in the tape-strips was subsequently extracted quantitatively and analyzed by liquid chromatography (see method below). The amount of BMV on each strip was then converted to a concentration at a specific depth into the SC. Although the gravimetric approach to determine the amount of SC removed on each strip is labor-intensive, the method remains, for the moment, the 'gold-standard' against which alternative procedures, such as those based on protein quantification^{27,28}, are calibrated.

To calculate the total thickness of the SC (L), the same tape-stripping procedure was performed at an adjacent, untreated skin area with measurements of TEWL after each tape-strip²⁶. The amount of SC removed on each tape was again determined gravimetrically and converted into a SC thickness removed on each strip. It was then possible to calculate the total thickness of the SC from the x-intercept of a graph of $TEWL^{-1}$ versus the cumulative thickness of SC removed²⁶. Knowing the SC thickness of each subject made it possible to express all BMV concentration profiles as a common function of the relative position (depth) into the SC, greatly facilitating objective comparison of the results^{15,29}.

A second DPK study was then carried out according to the FDA Draft Guidance⁶. Six human volunteers (all female, aged 25-32 years), in good general health and with no history of dermatological disease, participated in these experiments. The same infinite volume of either the MCT or ME formulation (containing drug at 80% of its saturation level) was placed in a 1.2 cm diameter Hill Top Chamber[®] and affixed via an adhesive tape to the volar forearm. A maximum of six chambers, three with the MCT formulation and three with the ME, were applied (Figure 2). For drug uptake, the formulations were applied to the left forearm and the SC samples were collected from each site immediately after removal of the chambers at 2, 4 and 6 hours. To assess drug elimination, the formulations were applied to the right forearm, and maintained in place for 6 hours. All formulations were then removed (using dry paper swabs) and SC samples were taken after a further 2, 6 and 24 hours.

Each treated skin site was initially tape-stripped 12 times; additional tape-strips were taken, if necessary, until the value of transepidermal water loss (TEWL) was ~4-fold greater than the pre-stripping value measured at an adjacent skin site and untreated with either formulation. Periodic measurements of TEWL, before and after the stripping process, were performed. A 4-fold increase in the TEWL value should have ensured that

at least 75% of the SC was removed at each skin site¹⁵. The first tape-strip was discarded to avoid potential residual drug contamination. The drug on the remaining tape-strips was subsequently extracted and analyzed by HPLC (see method below). The total amount of BMV recovered from the tape-strips was expressed in micrograms per square centimeter ($\mu\text{g cm}^{-2}$).

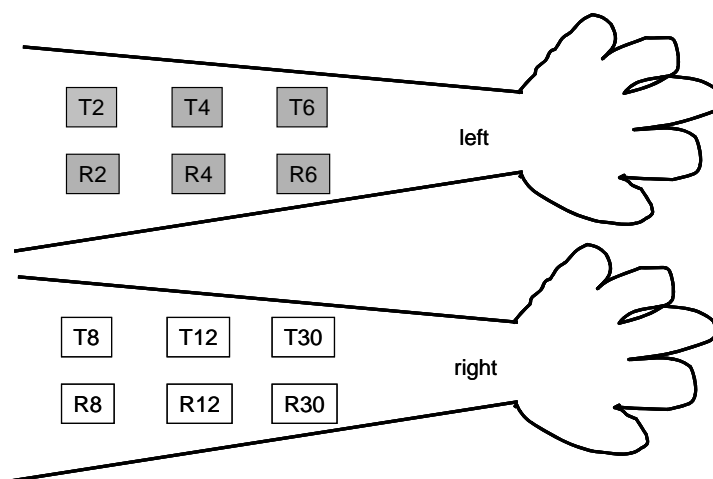


Figure 2: Dermatopharmacokinetic application scheme according to the Draft Guidance⁶. T = test vehicle (ME), R = reference vehicle (MCT). Uptake phase 2 to 6 hours ; elimination phase 8 to 30 hours .

Extraction and analysis of BMV

Each tape from the first DPK study was completely extracted by overnight shaking with 1.0 mL of 60:40 (v/v) acetonitrile/water (Sigma-Aldrich, Steinheim, Germany). Tapes from the second set of measurements were extracted in groups of 5 or fewer. Validation of the extraction procedure involved spiking tape-stripped samples of untreated SC with a known quantity of BMV. Recovery was $96.9 \pm 3.4 \%$ ($n = 5$). BMV in the various samples was quantified by high-performance liquid chromatography (HPLC) analysis (Dionex, Munich, Germany) using a Lichrospher[®] 100 RP-18 (4 x 125

mm) column (Hichrom, Reading, UK) with UV detection at 240 nm. The mobile phase was degassed acetonitrile/deionized water (60:40 v/v) and was delivered at a flow rate of 1 mL min⁻¹ in a 50-μL sample loop. The retention time of BMV at 25°C was ~3.8 minutes. BMV was determined using the area under the curve method and calibration plots were generated with the neat compound (R² = 0.999). The limit of quantification was 0.1 μg mL⁻¹.

Statistics

Statistical data analysis was performed using paired two-tailed Student's t-tests and a one- and two-way Analysis of Variance (ANOVA). P-values less than 0.05 were considered statistically significant.

Analysis of the concentration profile data

The SC distribution profiles of BMV (i.e. drug concentration (C_x) as a function of position (x) within the SC and time (t)) were fitted to the appropriate solution of Fick's second law of diffusion³⁰:

$$C_x = KC_v \left[1 - \frac{x}{L} - \frac{2}{\pi} \sum_{n=1}^{\infty} \frac{1}{n} \sin(n\pi \frac{x}{L}) \exp(-\frac{D}{L^2} n^2 \pi^2 t) \right] \quad \text{Eq. 1}$$

where C_v is the BMV concentration in the vehicle, K is the apparent partition coefficient of BMV between the SC and the applied vehicle, and D is the diffusivity of the drug in the SC of total thickness L.

The analysis assumes the following boundary conditions: (i) at the skin surface (x = 0), for the entire duration of the experiment, the BMV concentration is K•C_v; (ii) at t = 0, the SC contains no drug; and (iii) at the inner surface of the SC (x = L), perfect 'sink' conditions exist for the drug.

Fitting the experimental data from the first set of tape-stripping experiments to Eq. 1 (using GraphPad Prism[®] 4.03 Software, San Diego, CA, USA) allowed estimates of K and D/L² to be derived. The latter has units of a first-order rate constant (time⁻¹), and is a 'classic' diffusion parameter derived from these experiments³¹. In terms of topical BA, K reports on the extent of drug delivery, while D/L² reflects information about the rate of uptake into the SC. Integration of Eq. 1 yields the area under the drug concentration profile (area under the curve, AUC) in units of amount per volume:

$$AUC = \int_0^1 C_x d\left(\frac{x}{L}\right) = KC_v \left[\frac{1}{2} - \frac{4}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left(-\frac{D}{L^2} (2n+1)^2 \pi^2 t\right) \right] \quad \text{Eq. 2}$$

As the SC thickness (L) was independently determined, it was further possible to evaluate the total amount (A) of BMV in the barrier in units of amount per unit area via the simple conversion:

$$A = AUC \cdot L \quad \text{Eq. 3}$$

In this way, from the K and D/L² values in the initial series of experiments, it was possible to predict the values of A as a function of time in the second set of DPK measurements.

Finally, using the "elimination phase" (6-30 hours) results from the second series of tape-stripping experiments, an effective elimination rate constant (k_e) of BMV from the SC was determined assuming a mono-exponential, first-order decay:

$$A_{6-30} = A_6 \cdot \exp[-k_e \cdot (t - 6)] \quad \text{Eq. 4}$$

where A₆ is the amount per unit area of drug in the SC at 6 hours.

Results

Influence of vehicle and dose duration on skin blanching

The mean blanching response versus time profiles, as a function of dose duration and vehicle, are shown in Figure 3. BMV was applied at the same thermodynamic activity (80% of saturation) in each vehicle. The chromameter readings were baseline-adjusted and untreated control site corrected and are expressed as Δa values. The blanching response increased with time for all formulations post-removal reaching a maximum 4-6 hours later. It would appear, therefore, that drug taken up into the SC during the application period was still being released to the site of vasoconstrictive response post-removal of the delivery system, a manifestation of the phenomenon often referred to, in the literature, as a 'reservoir' effect^{2,3,32-35}.

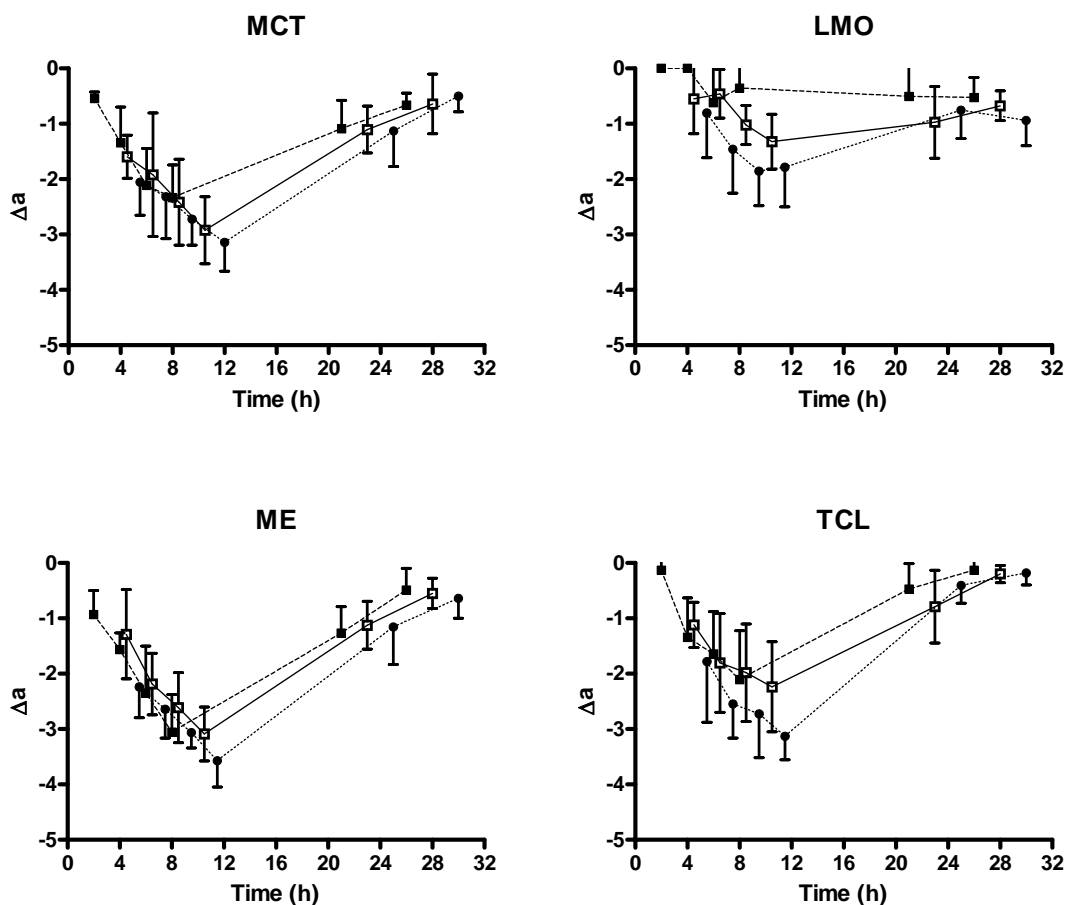


Figure 3: Corticosteroid-induced skin blanching. Vasoconstriction response (Δa) versus time profiles provoked by BMV delivered in MCT, LMO, ME and TCL following dose durations of 2-h ■, 4-h □ and 6-h ● (mean \pm SD; $n = 6$). For clarity of viewing, some data have been slightly displaced on the time-axis and only +SD and -SD are shown for the 2-h and 6-h results, respectively.

The skin blanching response increased with increasing dose duration. Analysis of variance indicated that both the vehicle and the dose duration had a significant influence on the AARC values ($P < 0.05$, two-way ANOVA). Figure 4 summarizes these results and shows clearly that the drug delivered from LMO shows the lowest pharmacodynamic response at all investigated dose durations. On the other hand, the blanching response

profiles induced by BMV delivered from MCT, ME and TCL were similar, and not significantly different from each other. While these latter findings would be consistent with the anticipated equivalence of delivery from vehicles containing the drug at similar thermodynamic activities, the poorer performance of LMO, which was not seen in earlier work³⁶, suggests that this formulation is retarding BMV transport in some way. However, because these conclusions are based on measurements of a pharmacodynamic response, which is known to be saturable, an independent approach (namely, the tape-stripping component of this work described below) to evaluate drug delivery is desirable.

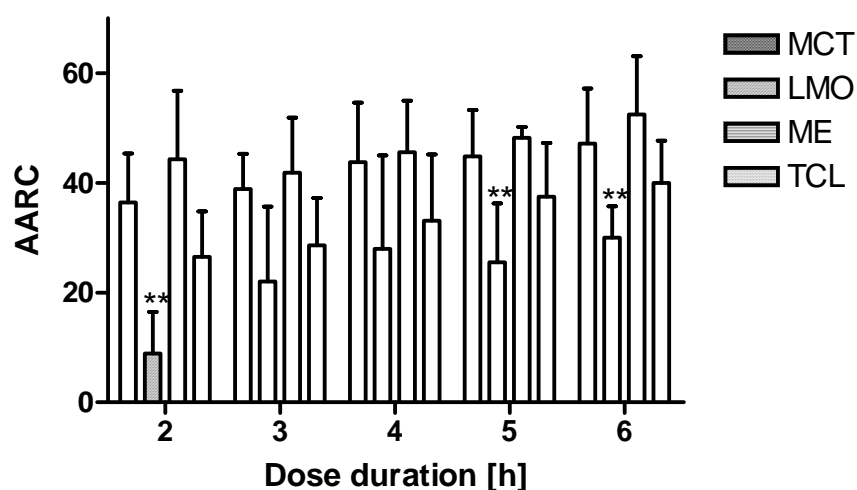


Figure 4: Skin blanching depends on steroid formulation. AARC values derived from the vasoconstriction response profiles as a function of dose duration and vehicle (mean \pm SD, $n = 6$). Significant differences ($P < 0.01$) relative to the reference formulation (MCT) are shown by the double asterisks.

Influence of vehicle on drug uptake into SC

Uptake of BMV from the different vehicles into the SC, determined by tape-stripping, yielded the results collected in Figure 5 for MCT (the reference formulation), ME and TCL. Data from LMO could not be obtained because the levels of drug extracted

from the tape-strips were below the BMV assay's limit of quantification. Fitting Eq. 1 to the profiles generated individual values of K and D/L^2 parameters, the means (\pm SD) of which are in Table 1. AUC values were calculated from these parameters using Eq. 2 and are also included in Table 1. BMV delivery was significantly different between all formulations with $TCL > ME > MCT$ (ANOVA, $p < 0.05$). In terms of AUC, TCL outperformed MCT by a factor of more than 60-fold, while ME achieved a 5-fold improvement relative to the reference formulation. Interestingly, the values of K and D/L^2 deduced from the concentration profiles were not significantly different between MCT, ME and TCL (Table 3). In contrast, the deduced saturation concentrations of BMV in the SC ($C_{s,SC}$) were highly vehicle-dependent implying that components of the ME and TCL formulations had also been taken up into the SC in sufficient quantities to alter the drug's solubility in the barrier. Such behavior has been previously reported for Transcutol®³⁷⁻³⁹ and for other cosolvents, such as propylene glycol^{22,40}.

It is noteworthy that the values of K derived from these experiments are not far removed from unity. We have observed not dissimilar behavior in recent work as well¹⁹⁻²². While an unequivocal explanation for this phenomenon cannot be offered at this time, it is possible that the formulation almost 'overwhelms' the outermost layer of the SC, such that partitioning occurs between two rather similar phases as a result. Further experiments are required to shed further light on this issue. The similarity in the values of D/L^2 indicate that excipients from the formulations did not significantly promote or retard BMV diffusion across the SC (as has been seen before, for example, when terbinafine was administered in a vehicle containing the known penetration enhancer, oleic acid¹⁶). The absolute values of D/L^2 for BMV are somewhat smaller than those which have been reported for ibuprofen¹⁹⁻²², consistent with the larger molecular weight of the corticosteroid.

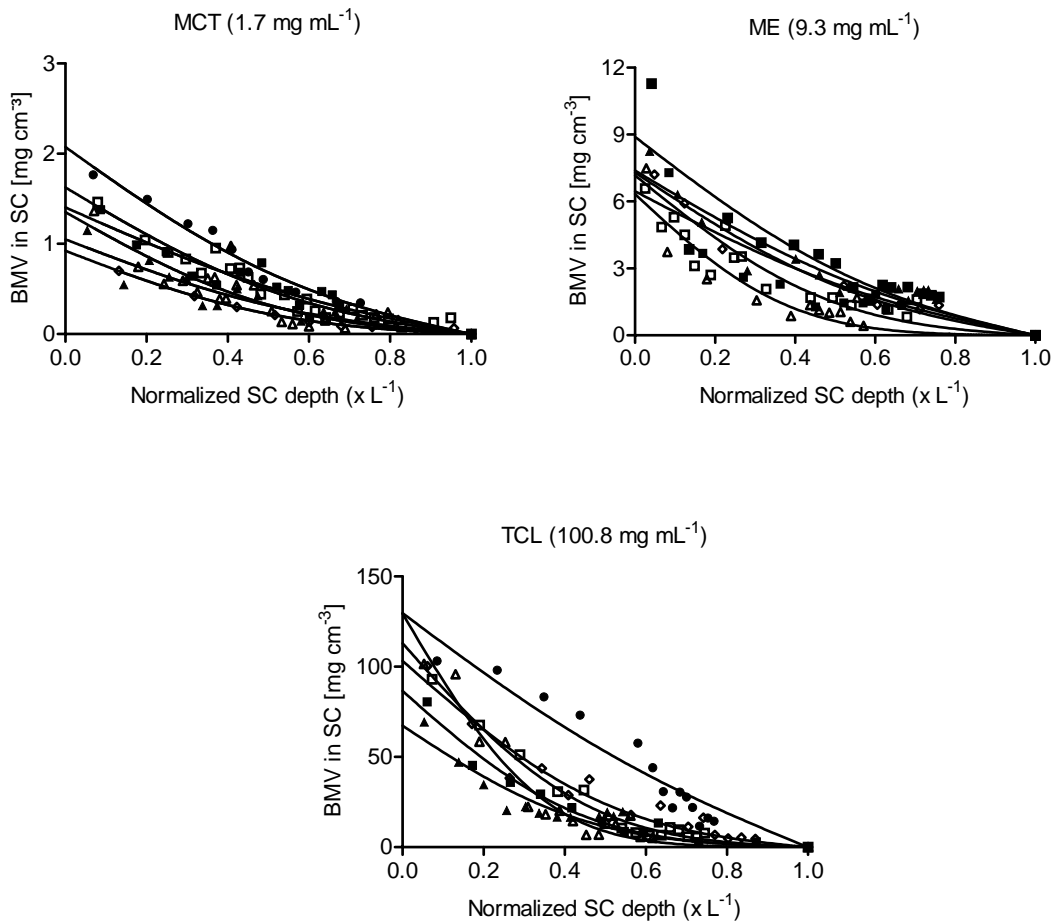


Figure 5: Dermatopharmacokinetic profiles depend on steroid formulation. BMV concentration versus normalized depth into the SC following a 2-hour application of the drug at 80% of saturation in three different vehicles: MCT, ME and TCL. The individual profiles from 6 volunteers are shown, together with the best fits to the data of Eq. 1.

Table 3: Tape-stripping experimental results (mean \pm SD, $n = 6$) for BMV delivered from 4 vehicles following a 2-hour application. The drug concentration in each vehicle (C_v) equalled 80% of its saturation level.

Vehicle	C_v [mg mL ⁻¹]	K^a	D/L^2 [h ⁻¹] ^a	$C_{s,SC}$ [mg mL ⁻¹] ^b	AUC [mg cm ⁻³] ^c	A [μg cm ⁻²] ^d
MCT	1.7	0.83 \pm 0.24	0.058 \pm 0.013	1.68 \pm 0.49*	0.51 \pm 0.18*	0.77 \pm 0.40*
ME	9.3	0.78 \pm 0.10	0.056 \pm 0.021	9.13 \pm 1.15*	2.61 \pm 0.67*	3.73 \pm 1.40*
TCL	100.8	1.04 \pm 0.24	0.042 \pm 0.028	131 \pm 30.8*	32.5 \pm 13.3*	38.0 \pm 17.5*
LMO	0.0017	n.d. ^e	n.d. ^e	-	n.d. ^e	n.d. ^e

^a Obtained by fitting experimental data to Eq. 1.

^b $C_{s,SC} = K \cdot C_{s,V}$.

^c From Eq. 2 using the corresponding value of K and D/L^2 .

^d From Eq. 3 using the corresponding, independently-determined values of L .

^e Not determined as BMV could not be quantified in tape-strip extracts.

* Significantly different from each other ($p < 0.05$).

Influence of drug concentration applied on skin blanching

The first series of vasoconstrictor experiments, taken together with the tape-stripping results, intimated that the pharmacodynamic response had become saturated when BMV was applied at 80% of its maximum thermodynamic activity. Therefore, a second set of experiments was performed, using the MCT and ME formulations, to examine skin blanching when the drug was administered at lower doses. A single dose duration of 4 hours was chosen and the vasoconstriction was followed over the next 24 hours. The results show that the response is indeed concentration-dependent, as has been previously suggested⁴¹. Figure 6 expresses the data in terms of the AARC as a function of both the BMV concentration in the vehicle and its degree of saturation.

Because the concentrations employed vary over three orders of magnitude, the data have been plotted with a logarithmic x-axis in the normal way. It is clear that the AARC did not increase over the last log unit of concentration (from 1 to 10 mg mL⁻¹), suggesting that the response was indeed saturated; in contrast, up to ~1 mg mL⁻¹, AARC increased log-linearly with concentration.

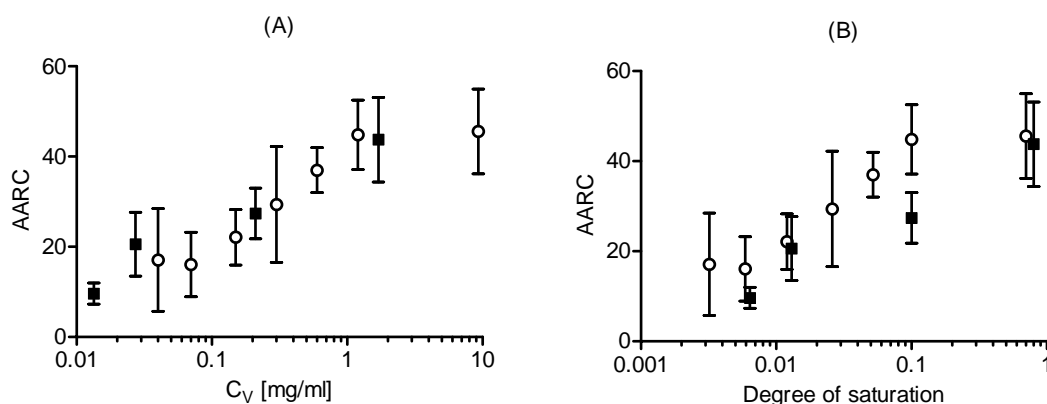


Figure 6: Skin blanching dose-response behaviour. AARC values derived from the vasoconstriction profiles as a function of (A) BMV concentration (C_V), and (B) degree of saturation in the vehicles studied (MCT (■) and ME (o)) following a 4-hour application (mean \pm SD, $n = 6$).

Influence of drug concentration applied on uptake into the SC

It was then logical to determine whether BMV delivery into the SC showed a concentration dependence and to determine the nature of the relationship. Again, using the MCT and ME vehicles, the SC uptake of drug was evaluated as a function of concentration in the formulation. The results, following a 2-hour application are summarized in Figure 7 (and Figure 5) and Table 4.

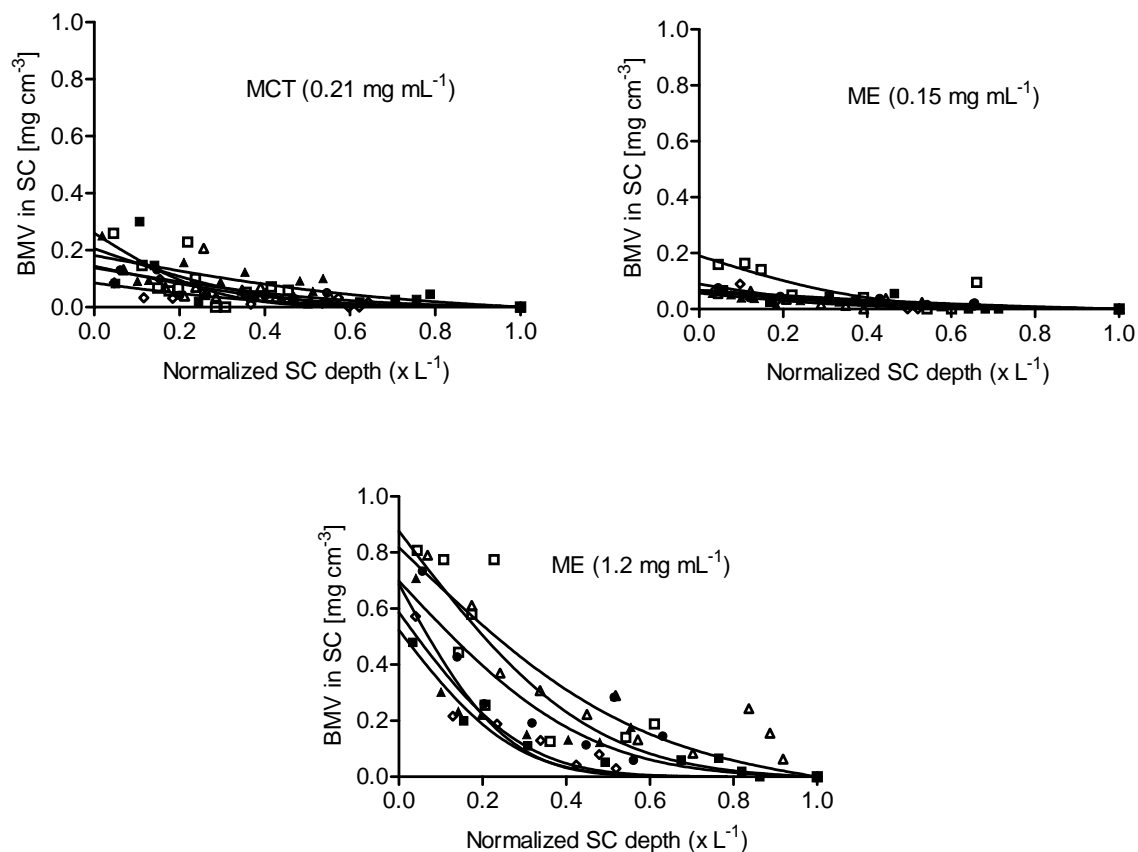


Figure 7: Dermatopharmacokinetic dose-response behavior. BMV concentration versus normalized depth into the SC following a 2-hour application of the drug in MCT and ME at different concentrations. The individual profiles from 6 volunteers are shown, together with the best fits of the data to Eq. 1.

The lowest concentrations considered were those that resulted in quantifiable amounts of drug in the tape-strips; for this reason, only one other MCT formulation was studied while two others were possible for ME.

Table 4: Tape-stripping experimental results (mean \pm SD, $n = 6$) for BMV delivered from MCT and ME following a 2-hour application. The drug concentrations in each vehicle (C_v) were modified to significantly alter the BMV thermodynamic activity.

Vehicle	C_v [mg mL ⁻¹]	Degree of $C_{s,v}$	K^a	D/L^2 [h ⁻¹] ^a	$C_{s,sc}$ [mg mL ⁻¹] ^b	AUC [mg cm ⁻³] ^c
MCT	1.7	0.80	0.83 \pm 0.24	0.058 \pm 0.013	1.68 \pm 0.49	0.51 \pm 0.18
	0.21	0.10	0.80 \pm 0.29	0.036 \pm 0.020	1.69 \pm 0.61	0.05 \pm 0.02*
ME	9.3	0.80	0.78 \pm 0.10	0.056 \pm 0.021	9.13 \pm 1.15	2.61 \pm 0.67
	1.2	0.10	0.58 \pm 0.11	0.025 \pm 0.016	6.81 \pm 1.30	0.17 \pm 0.08*
	0.15	0.013	0.59 \pm 0.33	0.042 \pm 0.024	6.95 \pm 3.96	0.03 \pm 0.01*

^a Obtained by fitting experimental data to Eq. 1.

^b $C_{s,sc} = K \cdot C_{s,v}$.

^c From Eq. 2 using the corresponding value of K and D/L^2 .

* Significantly different from the corresponding value at $0.80 \cdot C_{s,v}$ ($p < 0.05$).

It is first noted that the estimated values of K and D/L^2 were not significantly different from those derived from the results for the vehicles which contained BMV at 80% of its saturation level (Table 4). In contrast, the AUC values were decreased when the drug was applied at lower concentrations, with the reductions observed being more or less proportional to the corresponding change in BMV thermodynamic activity. For example, an 8-fold decrease in BMV level in the MCT vehicle resulted in an approximately order of magnitude reduction in AUC; similarly, when the drug concentration in ME was lowered by ~ 60 -fold, the AUC decreased by about a factor of 80. It follows that, unlike the skin blanching response, drug uptake into the SC from a vehicle, in which the compound is below its solubility, is not saturable.

This point is reinforced when the AARC derived from the vasoconstriction response profile is plotted against the SC uptake AUC determined by tape-stripping (Figure 8). When all relevant data are included (i.e., one concentration for TCL, two for MCT and three for ME), it is clear that the delivery of BMV into the SC is affected by both the thermodynamic activity of the drug and by specific vehicle-skin interactions. No evidence for saturation of the barrier can be identified. In contrast, despite a nearly three order change in AUC, the vasoconstriction response changed by less than a factor of 10 and appears saturable. This may be due to a straightforward pharmacological effect; alternatively, the perceived saturation may be the result of the limited solubility of the steroid in the region where the receptors are located. However, distinguishing these (or other possibilities) is beyond the scope of the present work.

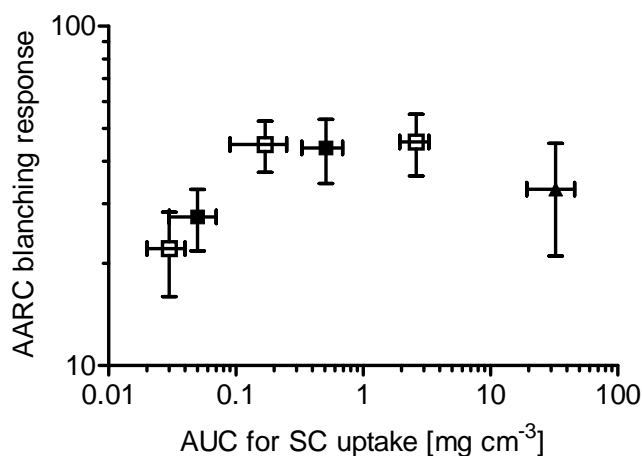


Figure 8: Skin blanching versus dermatopharmacokinetics. AARC values derived from the vasoconstriction profiles (determined after a 4-hour dose duration) plotted against the corresponding AUC values for SC uptake determined by tape-stripping after a 2-hour application. Data for BMV delivered from MCT (■), ME (□) and TCL (▲) are shown (mean ± SD, n = 6).

Comparison with Draft Guidance DPK protocol

Finally, Figure 9A compares the DPK profiles of BMV delivered from MCT and ME following the Draft Guidance protocol⁶. Significant differences in the amount of drug in the SC were observed at all time points between the two vehicles. ME was clearly the more effective formulation. At 6 hours, SC uptake of BMV from the ME was about 6 $\mu\text{g cm}^{-2}$, compared to only 1 $\mu\text{g cm}^{-2}$ from the MCT formulation. The corresponding BA parameter A_{max} reflects this observation, and differed significantly between the two vehicles (1.15 ± 0.32 and $7.92 \pm 1.50 \mu\text{g cm}^{-2}$ for MCT and ME, respectively; $P < 0.05$). The ratio of AUC values (ME/MCT) was 5.05 ± 1.25 . The T_{max} values for the two vehicles (5.7 ± 0.8 h for MCT, 4.3 ± 2.0 h for ME) were not significantly different, on the other hand. When the elimination phase of the DPK profiles was fitted to Eq. 4, no difference in the effective elimination rate constant (k_e) values was found: 0.04 ± 0.02 and $0.06 \pm 0.02 \text{ h}^{-1}$ for MCT and ME, respectively. Furthermore, it is worth noting that these absolute values are numerically very similar to the D/L^2 results reported in Tables 3 and 4.

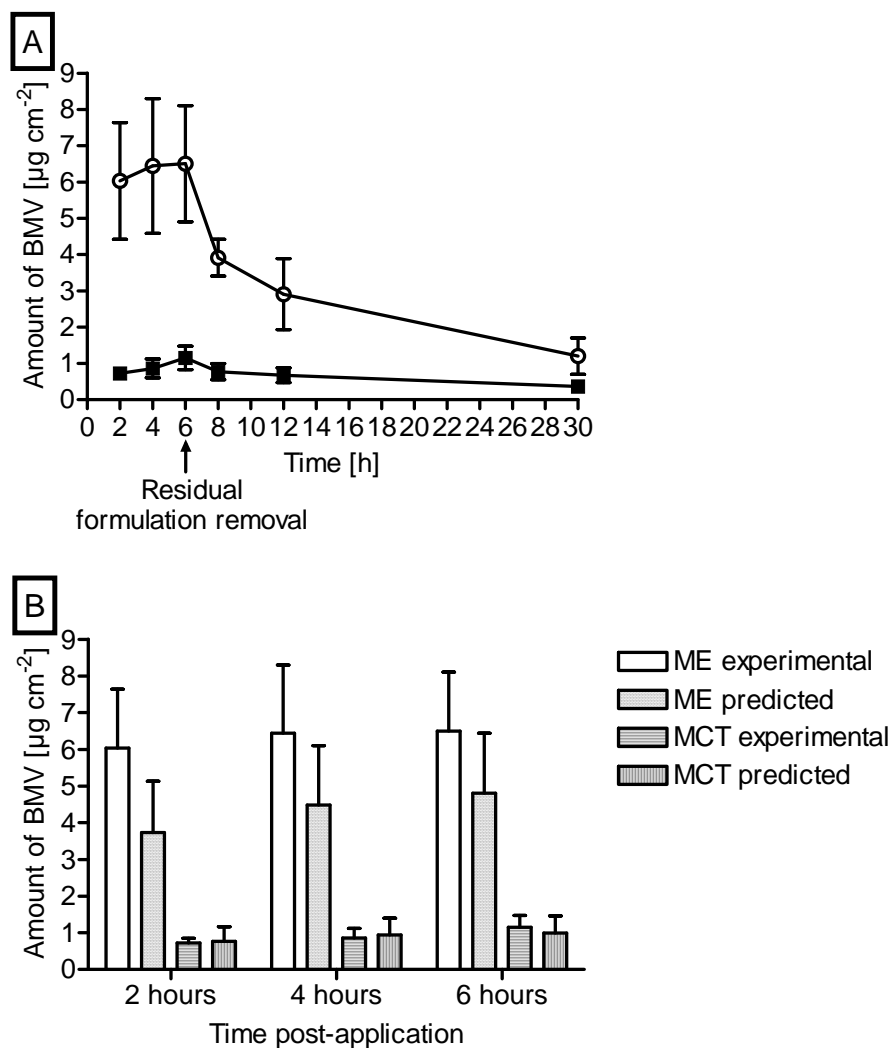


Figure 9: Refined dermatopharmacokinetic approach for topical BA/BE assessment. **A** - DPK profiles (amount of BMV versus time) of reference (MCT ■) and test (ME o) formulations (mean \pm SD, $n = 6$). For the uptake phase, formulations were removed at 2, 4 and 6 hours. For the elimination phase, formulations were maintained to the skin for 6 hours, subsequently removed, and the SC was stripped after a further 2, 6 and 24 hours. **B** - Experimentally measured and predicted (using Eqs 2 and 3, and the values of K and D/L^2 , with their respective variances, determined from the first series of tape-stripping studies) amounts of BMV in the SC, delivered from ME and MCT, during the uptake phase of the DPK experiment.

Interestingly, it was possible to use the values of K and D/L^2 derived from the 2-hour experiment in the first series of tape-stripping studies to predict the amounts (A) of BMV in the SC from Eqs. 2 and 3 during the uptake, or 'absorption', phase of the experiment (i.e., up to 6 hours when the formulation was removed). Figure 9B compares the experimental and predicted values, which agree very well for MCT, and differ insignificantly (by less than a factor of 2) for ME. As suggested by recent work²¹, therefore, it may be possible to significantly simplify a DPK-type bioequivalence study, in terms of the number of time points required to characterize uptake and elimination phases, relative to that outlined in the original Draft Guidance⁶.

In conclusion, this work demonstrates that the evaluation of topical BA is complex and may be sensitive to the methodology employed. While the vasoconstrictor assay has been employed extensively for corticosteroids, the saturable nature of the response means that results from such experiments must be analyzed with care (as indeed they have been in most occasions in the past). The tape-stripping, or DPK approach, would appear to offer a reliable metric with which to quantify transfer of drug from the vehicle to the SC. The critical validation of this measure to clinical outcome remains a long-term objective that will probably be achieved on a case by case (or drug class by drug class) basis. Methodological questions remain here as well; for example, the SC recoveries are dependent upon the surface cleaning procedure at the end of the application period being both efficient and benign (i.e., not encouraging penetration). Further work is essential to explore this question, in particular the potential significance of formulation which may become entrapped in skin 'furrows' and erroneously considered to be absorbed.

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